

## REMARKS

An Office Action was received for the instant case on March 22, 2007. In that action, claims 1, 3, and 5-20 were pending. The Examiner has rejected claims 1, 3, and 5-20. After entry of the instant amendments, claims 1, 3, 5, 12, and 13 are amended. Reconsideration of the Examiner's rejections is respectfully requested in view of the instant claim amendments and arguments provided hereinbelow.

### Informalities

The Examiner has objected to claim 3 for the recitation of "an domesticated" in place of "a domesticated". The Applicants have appropriately amended claim 3. Reconsideration is respectfully requested.

### Rejection under 35 U.S.C. § 112, 2<sup>nd</sup> paragraph

The Examiner has rejected claims 1, 3 and 5-20 under 35 U.S.C. § 112, 2<sup>nd</sup> paragraph for being indefinite, contending that the claims are vague, indefinite, incomplete, and incomprehensible. The Examiner then specifically identified (discussed in more detail hereinbelow) the alleged deficiencies of the claims.

Before discussing the particular alleged deficiencies, Applicants would like to provide a general overview of the claimed invention. As will be known by the Examiner, in the face of complete sequencing of the genomes of a number of organisms, the problem remains to identify the particular genes that are associated with a given physiological trait. For example, the genes underlying many diseases have not been elucidated, despite the existence of the complete human genome. The invention disclosed and claimed in claims 1, 3, and 5-20 provides a solution to this problem, i.e., provides methods to identify particular genes associated with a given commercially or aesthetically relevant trait in a domesticated species. The method by which this identification is accomplished is to use known comparative genomics methods for identifying regions of low nucleotide diversity, to indicate candidate genes for such traits.

Regions of low nucleotide diversity can indicate that at the time a species went through an evolutionary "bottleneck" event, an especially low allelic diversity for a particular gene region existed. Such a region of extremely low allelic diversity can indicate that the particular nucleotide region was related to a trait that enabled the species' survival through the bottleneck event. As discussed elsewhere herein, a bottleneck event is a sharp reduction of a

breeding population's size to a few individuals, which reduces genetic diversity in general. For domesticated organisms, it is known that most domesticated organisms contain less genetic variation than their wild ancestors, probably a product of a small initial crop population (i.e., a bottlenecking event).

It is the inventor's insight that such known methods for identifying regions of low nucleotide diversity, for a domesticated species, can be a method for identifying genes relevant to commercially important traits. It is known that for maize (corn), for example, a small number of single-gene changes accounts for all of the differences between the presently domesticated maize plant and its wild ancestor, teosinte, and account for all of the profound differences in morphology between maize and teosinte (Wang, (1999), *Nature* 398:236-239). Accordingly, it is the inventor's insight that comparative genomics, e.g., using the existence of regions of reduced nucleotide diversity in the genes presumably critical to traits important in domestication to act as a signal to identify those genes, could be a useful tool to identify these genes. As discussed in the Specification, Paragraph 0047, these genes have been exceedingly difficult to identify through standard methods, yet the majority of those genes are likely to show evidence of an evolutionary bottleneck, imposed by domestication.

Applicants now turn to the particular deficiencies pointed out by the Examiner. With respect to Office Action item (a) (Office Action, page 2), the Examiner stated that the metes and bounds of the term, "commercially or aesthetically relevant trait" in claims 1, 3, 12, and 13 was vague and unclear because the application does not distinguish between a commercially relevant trait and a non-commercially relevant trait. Applicants, in response, point out that the applicant identifies several commercially relevant traits in the application, e.g., yield, short day length flowering, protein content, oil content, drought resistance, taste, ease of harvest or disease resistance (most relevant to plants) (see Paragraph 0043); fat content, protein content, milk production, time to maturity, fecundity, docility or disease resistance/susceptibility (most relevant to animals) (see Paragraph 0044). One of skill in the art can readily identify those traits that are commercially relevant, such as the ones listed in the application, and those that are not. Reconsideration is respectfully requested.

With respect to Office Action Item (b) (Office Action, page 2), the Examiner stated that the term "relevant trait" in claims 1, 3, 12, and 13 is vague and unclear, again, because the application does not distinguish between a relevant trait and a non-relevant trait. First, Applicant points out that "relevant trait" is used together with "commercially or aesthetically", and cannot be separated from those modifying terms. Accordingly, the relevant analysis is for "commercially or aesthetically relevant trait". Applicant respectfully

directs the Examiner's attention to the immediately preceding paragraph, where this phrase is more specifically discussed. Reconsideration is respectfully requested.

With respect to Office Action item (c), the Examiner contends that the term "evolutionary bottleneck" is vague and indefinite because the term is not clearly defined in the application and there is no clear art-recognized meaning for the term. (Claims 1, 3, 12, and 13). Applicant respectfully disagrees. Attention is respectfully directed to Paragraph 0069, where the Applicant defines an evolutionary bottleneck as "an event that causes a severe decline in the size of a population, leaving a very few individuals for some period, followed by an increase in the surviving population. Evolutionary bottlenecks result in decreased allelic diversity." A number of approaches have been used in the art for an analysis for the presence of an evolutionary bottlenecking event, as is described in Eyre-Walker et al. p. 4441, and further described in the Specification. In particular, methods to find genes whose allelic diversity has been constricted by an evolutionary bottleneck, as described in Paragraph 0007 of the instant application, include the methods discussed in Paragraphs 0047-0058. Responsive to the Examiner's rejection, and to provide additional clarity to the claims, Applicants have amended claims 1, 3, and 12 (claim 13 has been amended to be dependent from claim 1 and includes claim 1's limitations) to clarify that the presence of an evolutionary bottleneck is signaled by standard analytical methods for the same, e.g., assessing the number of nucleotide differences per site, assessing fraction of nucleotides shared between homologous sequences, and the silent site nucleotide diversity on the aligned homologous nucleotide sequences. Accordingly, the evolutionary bottleneck analysis is defined in terms of the standard methods in the art by which such bottleneck is detected. Reconsideration is respectfully requested.

With respect to Office Action item (d) (page 2 of Office Action), the Examiner considered the claim language "detecting a region of polynucleotide sequence for which the number of nucleotide differences/site indicates an evolutionary bottleneck" (claims 1 and 3) vague and indefinite, alleging that the application does not disclose the number of nucleotide differences/site is indicative of an evolutionary bottleneck. Applicant understands the Examiner's rejection to be related to the fact that Applicant does not describe a particular number or range for the number of nucleotide differences/site as being indicative of an evolutionary bottleneck. The Applicant respectfully directs the Examiner's attention to Paragraphs 0055 to 0058, in particular. In these paragraphs, Applicant teaches to one of skill in the art the methods that will be required to find a positive signal of a nucleotide region having been through an evolutionary bottleneck. As taught in this section of the application,

the "background" level of nucleotide diversity for a portion of the nucleotide sequence for that species will have to be determined, in order to be able to detect a region that has a lower level of nucleotide diversity indicating a nucleotide region that has been evolutionarily bottlenecked, and is thus a candidate sequence for a commercially or aesthetically important trait. Reconsideration is respectfully requested.

In item (e) of the Office Action (page 3 of the Office Action) (claims 1 and 3), the Examiner states that "may be identified" is vague because it is not clear if the trait is actually identified. Responsive to this rejection, in order to further clarify the claim, the claim has been modified to recite "a polynucleotide sequence which is or is suspected to be associated with a commercially or aesthetically relevant trait of said domesticated organism is identified". The language clarifies that what is being identified is a candidate sequence for a commercially or aesthetically relevant trait of an organism. Reconsideration is respectfully requested.

In item (f) and item (g) of the Office Action (page 3 of the Office Action), the Examiner stated that the terms  $\pi$  and  $\Pi$ , respectively, are not defined (claims 6 and 15). Applicant respectfully directs the Examiner's attention to Paragraph 0048, where Applicant notes that  $\pi$  is the number of nucleotide differences/site. For  $\Pi$ , it is known in the art that the capital letter ( $\Pi$ ) is used to denote the mean pairwise differences per sequence and the lower-case letter ( $\pi$ ) to refer to the mean pairwise difference per site. Reconsideration is respectfully requested.

In item (h) of the Office Action (page 3 of the Office Action) (claims 7 and 16), the Examiner stated that the recitation of "displays a signature of positive selection" is vague, alleging that the application does not define how to recognize a signature of positive selection. The Applicant respectfully directs the Examiner's attention to Paragraphs 0055 to 0058, in particular. In these paragraphs, Applicant teaches to one of skill in the art the methods that will be required to find a positive signal of a nucleotide region having been through an evolutionary bottleneck. As taught in this section of the application, the "background" level of nucleotide diversity for a portion of the nucleotide sequence for that species will have to be determined, in order to be able to detect a region that has a lower level of nucleotide diversity indicating a nucleotide region that has been evolutionarily bottlenecked, and is thus a candidate sequence for a commercially or aesthetically important trait. Reconsideration is respectfully requested.

In item (i) of the Office Action (page 3 of the Office Action), the Examiner states that claim 12 is "incomprehensible", alleging that it recites a method of identifying an agent by

identifying an agent. Responsive to this rejection, Applicant has amended claim 12 to recite step (a), contacting at least one candidate agent with a cell, model system or transgenic plant or animal that expresses a polynucleotide sequence that is an evolutionary bottleneck, and a step (b) assaying for a modulated function of the polynucleotide sequence, wherein the agent is identified by its ability to modulate function of the polypeptide encoded by the polynucleotide. Support for the instant amendments may be found generally throughout the specification and particularly in Paragraphs 0163 to 0176. Thus, an agent is identified by its ability to modulate a function of a polynucleotide sequence, not by "identifying an agent." Reconsideration is respectfully requested.

Rejection under 35 U.S.C. § 102(e) over U.S. Patent 6,274,319

The Examiner has rejected claims 1, 3, 9, 14, and 16-18 as being anticipated by Messier et al. (U.S. Patent 6,274,319). The Examiner alleges that '319 teaches a method of sequence comparison for identification of a bottleneck which is embraced by the instant claims.

Applicant traverses this rejection. It is respectfully submitted that the Examiner's assertion that '319 "teaches a method of sequence comparison for identification of a bottleneck" is incorrect. While '319 does teach a method for sequence comparison, '319 teaches methods to identify commercially or aesthetically relevant traits by finding genes with "evolutionarily significant" nucleotide changes. The term "evolutionarily significant" has a defined meaning to one of skill in the art, and refers to a specific set of algorithms providing for analysis of sequences to determine a ratio between the number of nonsynonymous substitutions and synonymous substitutions in homologous genes. The most common type of algorithm used for this type of analysis is a  $K_A/K_S$ -type method. '319 contains extensive discussion of evolutionarily significant analyses and  $K_A/K_S$ -methods, see in particular, Col. 7, lines 51 through Col. 8, line 11.

In contrast, the instant application teaches methods to identify commercially or aesthetically relevant traits by finding genes that were "evolutionarily bottlenecked" by using algorithms that search for low sequence diversity, which are distinct from the  $K_A/K_S$ -type methods. As discussed explicitly in the instant application at Paragraph 0104, detection of nucleotides showing evidence of evolutionary bottlenecks is distinct from methods to detect evolutionarily selected nucleotides, in that evolutionary bottlenecks are independent of whether the same region has been evolutionarily selected.

Applicant accordingly submits that not all the elements of the instant claims have been disclosed in '319, specifically, that '319 in fact lacks any teaching of using algorithms for identifying a bottleneck to compare genes in order to identify candidate sequences for commercially or aesthetically relevant traits. Accordingly, not all elements of the instant claims are taught by '319. Reconsideration of this rejection is respectfully requested.

Rejection under 35 U.S.C. § 102(b) over Eyre-Walker et al.

The Examiner has also rejected claims 1, 3, 9, 14, 16, and 18 under 35 U.S.C. § 102(b) as being anticipated by Eyre-Walker et al., 1998, (PNAS 95:4441). The Examiner contends that Eyre-Walker et al. teaches a method of sequence comparison for identification of a bottleneck, which is embraced by the claims.

Applicant traverses this rejection. Applicant notes that Eyre-Walker et al., in this paper, are focused on explanation for the high genetic diversity among modern day maize, when its strong morphological distinctiveness of modern day maize strains from its ancestral progenitor teosinte suggest a strong domestication bottleneck, occurring approximately 7500 years ago. See page 4445, first column. Eyre-Walker then uses the known algorithms for evolutionary bottlenecking and coalescent investigations and applies these algorithms to DNA samples at a particular genetic locus, the *Adh 1* locus, of 19 individual plants of various maize and maize related species. Eyre-Walker concludes from this work that such sequence diversity in modern day maize can be explained by a bottleneck of short duration and small size (a 20 individual founding size when the bottleneck is only 10 generations in length, for example).

Applicant notes that in the course of this work, Eyre-Walker in particular was at pains to select a genetic locus for bottleneck testing that has not been affected by selection, either directly or indirectly. *Adh 1* is unaffected by selection, and therefore, could be relied on as an indicator of overall nucleotide diversity in maize. Essentially, then, Eyre-Walker used Coalescent theory to provide a population genetic framework for past events, utilizing historical information accrued in DNA sequences (i.e., nucleotide diversity at a particular genetic locus).

The present invention, as claimed, differs from Eyre-Walker in that the present invention includes the step of selecting from the sequences compared between at least two organisms those sequences or regions which have comparatively low nucleotide diversity to identify candidate sequences. Eyre-Walker discloses applying methods to determine low nucleotide diversity to one locus only, i.e., *Adh 1*. No step of selecting candidate sequences

is disclosed. Eyre-Walker et al. employed "bottleneck" type algorithms in a different context, i.e., to examine a specific locus (*Adh 1*), which had previously been identified as a gene which is merely indicative as indicator of overall nucleotide diversity in maize. *Adh 1* is taught by the reference as a gene region to test, but is **not** a gene region that is a candidate for a commercially or aesthetically relevant gene, as is required by the instant claims. Eyre-Walker et al is respectfully submitted to merely exemplify the prior art use of evolutionary bottlenecking type algorithms for (a) analyzing a known protein in order to determine whether said protein has been the subject of evolutionary bottlenecking, or (b) as a tool to estimate the approximate time of the bottleneck (as in Eyre-Walker et al.)

Thus, it is respectfully submitted that the cited art does not teach the instant method, as claimed, which is directed to methods to identify particular genes associated with a given commercially or aesthetically relevant trait in a domesticated species. The method by which this identification is accomplished is to use known comparative genomics methods for identifying regions of low nucleotide diversity, to indicate candidate genes for such traits. Accordingly, not all elements of the claims are disclosed by reference cited, and as such, the reference is not a proper § 102(b) reference. Reconsideration is respectfully requested.

#### Closing Remarks

If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-5117, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-5117.

Respectfully submitted,

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